Kindly amend the application as follows:

IN THE SPECIFICATION

Please substitute new specification page 69, attached herewith as Appendix A, for former specification page 69. The amendment to the specification is illustrated in the bracket and underline format in Appendix B.

IN THE CLAIMS

Please substitute new claim pages 93-111, attached herewith as Appendix C, for former claim pages 93-109. The amendments to the claims are illustrated in the bracket and underline format in Appendix D.

REMARKS

Applicants have amended the specification at page 69 to recite the level of solubility for the crosslinked subtilisin crystals illustrated in Figure 8. As such, this amendment does not constitute new matter.

Claims 1-79 and 81-85 are pending in this application. Applicants have amended claims 1, 17, 18, 19, 54, 55 and 56 and the claims dependent therefrom, to recite that the crosslinked protein crystals claimed or recited therein are "between about 30% and about 80% as soluble as an uncrosslinked counterpart protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours". Support for this

amendment can be found in the specification, for example, in Example 13 on pages 68 and 69 and in Figure 8.

Applicants have amended claim 2 to recite that the change from concentrate to dilute form comprises dilution of the protein crystal in a solution. This amendment is for the purpose of clarity and does not add new matter. Applicants have amended claims 31 and 84 to remove a second recitation of "toxoid" within each claim. Applicants have also added claims 86-88. Support for these claims can be found in the specification as originally filed, for example, on page 14, line 32 - page 15, line 7. Applicants have amended claims 2-9 to recite that the change from concentrate to dilute form comprises dilution of the protein crystal in a solution. This amendment is made to clarify the language. Finally, applicants have amended claims 2, 10, 12, 14, 16, 23, 27, 30-36, 39, 45-47 and 52-53 to additionally depend from new claims 86-88.

None of these amendments constitutes new matter.

35 U.S.C. § 102(a)

Claims 1-44, 46-63, 76 and 81-85 stand "rejected under 35 U.S.C. § 102(a) as anticipated by Navia et al." (United States patent 5,618,710) ("Navia"). Specifically, the Examiner contends that "Navia et al disclose crosslinked protein crystals that are inherently capable of being changed to soluble form by one or more of the changes claimed. The present claims encompass crosslinked

protein crystals and methods for preparation thereof disclosed by Navia et al." Specifically, the Examiner points to Examples 4 and 9 of Navia as exemplifying conditions similar to those recited in Examples 18-20 and 22 of the instant specification. Applicants traverse, based on the claim amendments presented herein.

In order to expedite allowance of this application, applicants have amended claims 1, 17, 18, 19, and 54-56 (and the claims which depend therefrom) to recite that the crosslinked protein crystals claimed or recited therein display a particular solubility in PBS solution, i.e. they are "between about 30% and about 80% as soluble as an uncrosslinked counterpart protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours".

In contrast to the crosslinked protein crystals recited in the amended claims, which exhibit this particular measure of solubility in PBS solution, Navia is directed towards the "immobilization" of proteins through crosslinking. Column 3, lines 9-25. Immobilization is defined in Navia as referring to "the insolubilization of enzyme catalyst by attachment to, encapsulation of, or by aggregation into macrascopic (10^{-1} mm) particles." Column 2, lines 7-10. As Navia makes clear, in its crosslinked enzyme crystals, "the lattice interactions, when fixed by chemical cross-links, are particularly important in preventing denaturation, especially in mixtures of aqueous and non-aqueous solvents (Klibanov, A.M., Trends in Biochemical Sciences 14: 141-

144 (1998))." Column 10, lines 19-23. This insolubility of the Navia crosslinked protein crystals is in marked contrast to the crosslinked protein crystals claimed or recited in the amended claims, which demonstrate a specific degree of solubility in the aqueous buffer PBS (or, regarding added claims 86-88, a specific release rate far in excess of anything possible with the crystals of Navia). Prevention of crystal solubility is not a goal of the instant application but, instead, the invention is directed towards crosslinked protein crystals (and methods of producing those crystals), which display an intermediate level of crosslinking which results in the controlled dissolution characteristics also recited in the amended claims. As such, the amended claims recite a different type of crosslinked protein crystal than those exemplified in Navia. For example, Navia states that their rehydrated thermolysin crosslinked enzyme crystals "could be routinely stored for months at room temperature with no apparent loss of activity" and "[t]hermal stability and resistance to autolysis were demonstrated in thermolysin CLECs following incubation at 65 °C for five consecutive days (FIG. 3 and Table 10)." Navia, Column 39, lines 6-13. Similarly, Navia's urease crosslinked enzyme crystal of Example 9 demonstrates 94% activity after 4 days incubation in 50 mM sodium phosphate buffer pH 7.0 at 55 °C. Navia, Column 49, Table 23 (Example 9). This is particularly relevant because Example 9 is cited by the Examiner in the May 23, 2001 Office Action as describing crosslinking

conditions similar to that of the instant invention. However, as is clear based on this data, the urease crosslinked crystals of Example 9 exhibit a stability outside the range of that recited for the crystals of the instant invention.

35 U.S.C. § 103(a)

Claims 45 and 64-75 stand rejected under 35 U.S.C. § 103(a) as being "unpatentable over" Navia. Specifically, the Examiner contends that the disclosure of Navia teaches the crystals of the instant invention and, therefore, also teaches the uses and methods claimed in the instant invention. This contention is moot in view of the amended claims.

As described above, Navia does not teach the controlled dissolution crystals of the amended claims. Further, nothing in Navia suggests such crystals. In fact, Navia teaches away from such controlled dissolution crystals by focusing on the inherent stability of its crosslinked protein crystals and their ability to withstand harsh conditions (not to mention aqueous buffers, such as PBS). As described above, Navia is directed towards a novel means of immobilization of protein crystals, wherein such immobilization results in a heavily crosslinked protein crystal which is remarkably insoluble in aqueous solvents. In contrast, the crosslinked protein crystals of the amended claims demonstrate a specific level of solubility in the aqueous buffer PBS which, in turn, results in the recited controlled dissolution

characteristics. As such, the uses and methods based on the crosslinked protein crystals of the amended claims are not obvious in view of Navia.

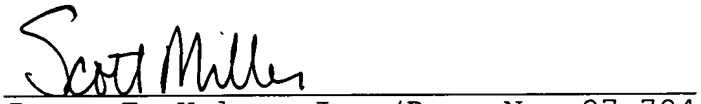
Claims 1-62, 76-79 and 81-85 stand rejected under 35 U.S.C. § 103(a) as being "unpatentable over" Navia, in view of Kausch et al. (United States patent 5,508,164), and if necessary in further view of Neville et al. (United States patent 5,066,490). Specifically, the Examiner contends that "[i]t would have been obvious to use a disulfide crosslinking agent as the crosslinking agent of Navia et al. to obtain reversible immobilization as disclosed by Kausch et al. If needed, Neville et al. would have further suggested a reversible crosslinking agent and that the agent can be other than a disulfide crosslinking agent." Applicants traverse.

As described above, Navia does not suggest the controlled dissolution crosslinked protein crystals of the amended claims. And neither Kausch et al. nor Neville et al. provide that suggestion, even combined with Navia. Accordingly, in combination, the cited documents do not render obvious any of applicants' claims.

CONCLUSION

Applicants request that the Examiner consider the foregoing amendments and remarks and pass this application to issue.

Respectfully submitted,



James F. Haley, Jr. (Reg. No. 27,794)
Margaret A. Pierri (Reg. No. 30,709)
Attorneys for Applicants
Scott D. Miller (Reg. No. 43,803)
Agent for Applicants
c/o FISH & NEAVE
1251 Avenue of the Americas
New York, New York 10020-1104
Tel.: (212) 596-9000

Crosslinker	Crosslinker Concentration	Crosslinking Time
GA	1.0%	1.5h
GA	0.25%	2h
GA	0.2%	2h
GA	0.15%	2h
NP/GA	0.1%/0.1%	5h/1.5h
411/GA	0.015%/0.035%	16h/1h
OA	0.2%	16h
OA	0.1%	16h
OA	0.05%	16h

The solubility profiles of the samples, shown in Figures 8 and 9, illustrate different rates of dissolution for the crosslinked crystals. For example, as shown in Figure 8, at 30°C the crosslinked crystals are about 30% to about 80% as soluble as an uncrosslinked counterpart protein crystal when stored in phosphate buffered saline solution for between about 100 and about 350 hours.

Example 14 - Reversible Crosslinkers - Disulfide Crosslinked Subtilisin Crystals

We prepared subtilisin crystals (30-40 μm average, 27 mg/ml in Na_2SO_4) as previously described for subtilisin crystallization.

We then crosslinked the crystals using one of the following crosslinkers:

1) Dimethyl 3, 3'-dithiobispropionimidate•HCl - (DTBP) (Pierce)
2) Dithiobis(succinimidylpropionate) - (DSP)(Pierce)
3) 3, 3'- Dithiobis (sulfosuccinimidylpropionate) - (DTSSP) (Pierce).

Crosslinking was carried out in 15 ml neoprene screw cap tubes by placing 740 μl of subtilisin crystal slurry (20 mg) in 9.26 ml of buffer (25 mM $NaCO_3$/50 mM $NaHCO_3$, pH 8.0). One crosslinker was

We Claim:

1. (Twice amended) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that:

a) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

b) said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form and releasing between about 0.1% and about 100% of crystalline protein as soluble protein per day upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.

2. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said change from concentrate to dilute form comprises dilution of said protein crystal in a solution.

3. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises an increase or decrease in salt concentration.

4. (Amended) The crosslinked protein crystal according to claim 3, wherein said dilution of said protein crystal in a solution comprises a decrease in salt concentration.

5. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises an increase or decrease in water concentration.

cn't B2

6. (Amended) The crosslinked protein crystal according to claim 5, wherein said dilution of said protein crystal in a solution comprises an increase in water concentration.

Rule 3

7. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises an increase or decrease in organic solvent concentration.

8. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises a decrease in detergent concentration.

9. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution comprises a decrease in protein concentration.

Rule 4

10. The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said change from concentrate to dilute form comprises a change in concentration of all solutes from about 2-fold to about 10,000-fold.

11. The crosslinked protein crystal according to claim 10, wherein said change from concentrate to dilute form comprises a change in concentration of all solutes from about 2-fold to about 700-fold.

12. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said change in pH comprises a change from acidic pH to basic pH.

13. The crosslinked protein crystal according to claim 1, wherein said change in pH comprises a change from basic pH to acidic pH.

14. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said change in temperature comprises an increase or decrease in temperature.

15. The crosslinked protein crystal according to claim 14, wherein said change in temperature is an increase in temperature from a low temperature between about 0°C and about 20°C to a high temperature between about 25°C and about 70°C.

16. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said active form of said protein is a form which is active against macromolecular substrates.

17. (Twice amended) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that:

a) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart

of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

b) said protein crystal has a half-life of activity under storage conditions which is greater than at least 2 times that of the soluble form of the protein that is crystallized to form said crystal that is crosslinked and activity similar to that of the soluble form of the protein under conditions of use and which releases between about 0.1% and about 100% of crystalline protein as soluble protein per day.

18. (Twice amended) A crosslinked protein crystal, said protein crystal being crosslinked by a multifunctional crosslinking agent such that:

a) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at 30°C for between about 100 and about 350 hours; and

b) said protein crystal is capable of releasing its protein activity at a controlled rate of between about 0.1% and about 100% of crystalline protein as soluble protein per day upon exposure to a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in pH, change in solute concentration, change in temperature, change in chemical composition, change in shear force acting upon the crystal and combinations thereof.

19. (Amended) The crosslinked protein crystal according to claim 18, wherein said controlled rate of releasing protein activity is determined by a factor selected from the group consisting of: the degree of crosslinking of said crosslinked protein crystal, the

length of time of exposure of protein crystal to the crosslinker, the amino acid residues involved in the crosslinks, whether the crosslinker is homobifunctional or heterobifunctional, the rate of addition of the crosslinking agent to said protein crystal, the nature of the crosslinker, the chain length of the crosslinker, the surface area of said crosslinked protein crystal, the size of said crosslinked protein crystal, the shape of said crosslinked protein crystal and combinations thereof.

21. The crosslinked protein crystal according to claim 18, wherein said crystal has a protein activity release rate between about 0.01% per hour and about 100% per hour.

22. The crosslinked protein crystal according to claim 18, wherein said crystal has a protein activity release rate between about 1% per minute and about 50% per minute.

23. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, said protein crystal being substantially insoluble and stable in a composition under storage conditions and substantially soluble and active under conditions of use of said composition.

24. The crosslinked protein crystal according to claim 23, wherein said composition is selected from the group consisting of cleaning agents, detergents, personal care compositions, cosmetics, pharmaceuticals, veterinary compounds, vaccines, foods, feeds, diagnostics and formulations for decontamination.

25. The crosslinked protein crystal according to claim 24, wherein said detergent is selected from the group consisting of powdered detergents, liquid detergents, bleaches, household cleaners, hard surface cleaners, industrial cleaners, carpet shampoos and upholstery shampoos.

Rwt-C12

26. The crosslinked protein crystal according to claim 24, wherein said cosmetic is selected from the group consisting of creams, emulsions, lotions, foams, washes, gels, compacts, mousses, sunscreens, slurries, powders, sprays, foams, pastes, ointments, salves, balms, shampoos, sunscreens and drops.

B
Rwt-C13

27. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, wherein said protein is an enzyme.

28. The crosslinked protein crystal according to claim 27, wherein said enzyme is selected from the group consisting of hydrolases, isomerases, lyases, ligases, transferases and oxidoreductases.

29. The crosslinked protein crystal according to claim 28, wherein said enzyme is selected from the group consisting of proteases, amylases, cellulases, lipases and oxidases.

Rwt-C14
B8

30. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, wherein said protein is selected from the group consisting of therapeutic proteins, cleaning agent proteins, personal care proteins, veterinary proteins, food proteins, feed proteins, diagnostic proteins and decontamination proteins.

31. (Twice amended) The crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, wherein said protein is selected from the group consisting of hormones, antibodies, inhibitors, growth factors, trophic factors, cytokines, lymphokines, growth hormones, nerve growth hormones, bone morphogenic proteins, toxoids and nutrients.

32. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, wherein said protein is selected from the group consisting of insulin, amylin, erythropoietin, Factor VIII, TPA, dornase-α, α-1-antitripsin, urease, fertility hormones, FSH, LSH, postridical hormones, tetanus toxoid and diptheria toxoid.

33. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, said crystal having a longest dimension of between about 0.01 μm and about 500 μm.

34. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, said crystal having a longest dimension of between about 0.1 μm and about 50 μm.

35. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, said crystal having a shape selected from the group consisting of: spheres, needles, rods, plates, rhomboids, cubes, bipryamids and prisms.

36. (Amended) A composition comprising a crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88, said composition being

selected from the group consisting of cleaning agents, detergents, personal care compositions, cosmetics, pharmaceuticals, veterinary compounds, vaccines, foods, feeds, diagnostics and formulations for decontamination.

37. The composition according to claim 36, wherein said detergent is selected from the group consisting of powdered detergents, liquid detergents, bleaches, household cleaners, hard surface cleaners, industrial cleaners, carpet shampoos and upholstery shampoos.

38. The composition according to claim 36, wherein said cosmetic is selected from the group consisting of creams, emulsions, lotions, foams, washes, gels, compacts, sunscreens, slurries, powders, sprays, foams, pastes, ointments, salves, balms, shampoos, sunscreens and drops.

39. (Amended) A protein delivery system, said system comprising crosslinked protein crystals according to any one of claims 1, 17, 18, or 86-88, and a delivery device.

40. The protein delivery system according to claim 39, wherein said protein is selected from the group consisting of: detergent enzymes, cosmetic proteins, pharmaceutical proteins, agricultural proteins, vaccine proteins and decontamination proteins.

41. The protein delivery system according to claim 40, said protein delivery system being a microparticulate protein delivery system.

42. The protein delivery system according to claim 41, wherein said microparticulate protein delivery system comprises crosslinked protein crystals having a longest dimension between about 0.01 µm and about 500 µm.

43. The protein delivery system according to claim 42, wherein said microparticulate protein delivery system comprises crosslinked protein crystals having a longest dimension of between about 0.1 µm and about 50 µm.

44. The protein delivery system according to claim 41, wherein said microparticulate protein delivery system comprises crosslinked protein crystals having a shape selected from the group consisting of: spheres, needles, rods, plates, rhomboids, cubes, bipryamids and prisms.

45. (Amended) A detergent formulation comprising a crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88.

46. (Amended) A controlled release formulation comprising a crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88.

47. (Amended) A pharmaceutical controlled release formulation comprising a crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88.

48. A pharmaceutical controlled release formulation comprising a crosslinked protein crystal, said protein crystal being crosslinked by a multifunctional crosslinking agent and said crystal being

substantially insoluble under storage conditions and capable of releasing its protein activity *in vivo* at a controlled rate.

49. The pharmaceutical controlled release formulation according to claim 47, said pharmaceutical being capable of administration by parenteral or non-parenteral routes.

50. The pharmaceutical controlled release formulation according to claim 49, said pharmaceutical being capable of administration by oral, pulmonary, nasal, aural, anal, dermal, ocular, intravenous, intramuscular, intraarterial, intraperitoneal, mucosal, sublingual, subcutaneous or intracranial route.

51. The pharmaceutical controlled release formulation according to claim 47, wherein said pharmaceutical is capable of administration by oral route and said crosslinked protein crystal is substantially insoluble under gastric pH conditions and substantially soluble under small intestine pH conditions.

52. (Amended) A vaccine comprising a crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88.

53. (Amended) A formulation comprising a crosslinked protein crystal according to any one of claims 1, 17, 18, or 86-88 said formulation being selected from the group consisting of tablets, liposomes, granules, spheres, microspheres, microparticles and capsules.

54. (Twice amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals:

a) are between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

cont B1

b) are characterized by the ability to change from insoluble and stable form to soluble and active form upon a change in their environment, and to release between about 0.1% and about 100% of crystalline protein as soluble protein per day, wherein said change is selected from the group consisting of change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystals and combinations thereof.

55. (Twice amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals are:

a) characterized by a half-life of activity under storage conditions which is greater than at least 2

times that of the soluble form of the protein that is crystallized to form said crystals that are crosslinked;

b) between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

c) characterized by an activity similar to that of the soluble form of the protein and which releases between about 0.1% and about 100% of crystalline protein as soluble protein per day under conditions of use.

56. (Twice amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals are:

cnt B11

a) characterized by being capable of releasing their protein activity at a controlled rate and which release between about 0.1% and about 100% of crystalline protein as soluble protein per day upon exposure to a change in their environment, said change being selected from the group consisting of: change in pH, change in soluble concentration, change in temperature, change in chemical composition, change in shear force acting upon the crystals and combinations thereof; and

b) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours.

57. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, comprising the step of reacting said protein crystals with said first crosslinking agent and said at least a second crosslinking agent at the same time or in sequence.

58. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein, prior to reacting protein crystals with said crosslinking agent, said method further comprises the step of crystallizing said protein.

59. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein the conditions sufficient to induce crosslinking are dependent upon a factor selected from the group consisting of: the degree of crosslinking of said crosslinked protein crystals, the length of time of exposure of protein crystals to the crosslinking agent, the rate of addition of the crosslinking agent to said protein crystal, the nature of the crosslinker, the chain length of the crosslinker, the surface area of said crosslinked protein crystals, the size of said crosslinked protein crystals, the shape of said crosslinked protein crystals and combinations thereof.

60. The method for producing crosslinked protein crystals according to claim 56, wherein said controlled rate of releasing protein activity is determined by a factor selected from the group consisting of: the degree of crosslinking of said crosslinked protein crystals, the length of time of exposure of protein crystals to the crosslinking agent, the rate of addition of the crosslinking agent to said protein crystals, the nature of the crosslinker, the chain length of the crosslinker, the surface area of said crosslinked protein crystals, the size

of said crosslinked protein crystals, the shape of said crosslinked protein crystals and combinations thereof.

61. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is a multifunctional crosslinking agent.

62. The method for producing crosslinked protein crystals according to claim 61, wherein said crosslinking agent is a bifunctional crosslinking agent.

63. The method for producing crosslinked protein crystals according to claim 61, wherein said crosslinking agent is selected from the group consisting of: glutaraldehyde, succinaldehyde, octanedialdehyde and glyoxal.

64. The method for producing crosslinked protein crystals according to claim 61, wherein said crosslinking agent is selected from the group consisting of: halo-triazines, halo-pyrimidines, anhydrides of aliphatic or aromatic mono- or di-carboxylic acids, halides of aliphatic or aromatic mono- or di-carboxylic acids, N-methylol compounds, di-isocyanates, di-isothiocyanates and aziridines.

65. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is an epoxide.

66. The method for producing crosslinked protein crystals according to claim 65, wherein said epoxide is selected from the group consisting of: neopentyl glycol

diglycidyl ether, ethylene glycol diglycidyl ether, di-epoxides, tri-epoxides and tetra-epoxides.

67. (Amended) The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is glutaraldehyde at a concentration of 0.0076% to 0.5% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with the crosslinking agent for a period of time between about 3 minutes and about 120 minutes.

68. The method for producing crosslinked protein crystals according to claim 67, wherein said crosslinking agent is glutaraldehyde at a concentration of 0.005% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with the crosslinking agent for a period of time between about 10 minutes and about 30 minutes.

69. The method for producing crosslinked protein crystals according to claim 67 wherein, prior to reaction with said protein crystals, said glutaraldehyde is pretreated by incubation at 60°C with a buffer for 1 hour.

70. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is glyoxal at a concentration of 0.01% to 1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with the crosslinking agent for a period of time between about 30 minutes and about 60 minutes.

71. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is octanedialdehyde at a concentration of 0.05% to 1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with the crosslinking agent for a period of time between about 30 minutes and about 16 hours.

72. The method for producing crosslinked protein crystals according to claim 71, wherein said crosslinking agent is octanedialdehyde at a concentration of 1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with the crosslinking agent for a period of time between about 1 hour and about 3 hours.

73. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is succinaldehyde at a concentration of 1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with the crosslinking agent for a period of time between about 30 minutes and about 3 hours.

74. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said first crosslinking agent is epoxide at a concentration of 0.01% to 4% in the slurry and said second crosslinking agent is glutaraldehyde at a concentration of 0.1% to 0.2% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting said protein crystals with said first crosslinking agent for a period of time between about 1 hour and about 72 hours and reacting said protein crystals with said second crosslinking

agent for a period of time between about 1 hour and about 5 hours.

75. The method for producing crosslinked protein crystals according to claim 74, wherein said first crosslinking agent is epoxide at a concentration of 0.01% in the slurry and said second crosslinking agent is glutaraldehyde at a concentration of 0.1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting said protein crystals with said first crosslinking agent for about 5 hours and reacting said protein crystals with said second crosslinking agent for about 1.5 hours.

76. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said protein is an enzyme.

77. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is a reversible crosslinking agent.

78. The method for producing crosslinked protein crystals according to claim 77, wherein said reversible crosslinking agent is a disulfide crosslinking agent.

79. The method for producing crosslinked protein crystals according to claim 78, wherein said disulfide crosslinking agent is a homobifunctional crosslinking agent or a heterobifunctional crosslinking agent.

81. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said enzyme is selected from the group consisting of

hydrolases, isomerases, lyases, ligases, transferases and oxidoreductases.

82. The method for producing crosslinked protein crystals according to claim 81, wherein said enzyme is from the group consisting of proteases, amylases, cellulases, lipases and oxidases.

83. The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said protein is selected from the group consisting of therapeutic proteins, cleaning agent proteins, personal care proteins, veterinary proteins, food proteins, feed proteins, diagnostic proteins and decontamination proteins.

B13

84. (Amended) The method for producing crosslinked protein crystals according to claim 83, wherein said protein is selected from the group consisting of hormones, antibodies, inhibitors, growth factors, trophic factors, cytokines, lymphokines, growth hormones, nerve growth hormones, bone morphogenic proteins, toxoids, vitamins and nutrients.

85. The method for producing crosslinked protein crystals according to claim 84, wherein said protein is selected from the group consisting of insulin, amylin, erythropoietin, Factor VIII, TPA, dornase-α, α-1-antitripsin, urease, fertility hormones, FSH, LSH, postridical hormones, tetanus toxoid and diptheria toxoid.

B14

86. (New) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form and releasing about 100% of

crystalline protein as soluble protein per day upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.

87. (New) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form and releasing about 100% of crystalline protein as soluble protein per hour upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.

cnt B14

88. (New) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form and releasing between about 1% and about 50% of crystalline protein as soluble protein per minute upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.

Crosslinker	Crosslinker Concentration	Crosslinking Time
GA	1.0%	1.5h
GA	0.25%	2h
GA	0.2%	2h
GA	0.15%	2h
NP/GA	0.1%/0.1%	5h/1.5h
411/GA	0.015%/0.035%	16h/1h
OA	0.2%	16h
OA	0.1%	16h
OA	0.05%	16h

The solubility profiles of the samples, shown in Figures 8 and 9, illustrate different rates of dissolution for the crosslinked crystals. For example, as shown in Figure 8, at 30°C the crosslinked crystals are about 30% to about 80% as soluble as an uncrosslinked counterpart protein crystal when stored in phosphate buffered saline solution for between about 100 and about 350 hours.

Example 14 - Reversible Crosslinkers - Disulfide Crosslinked Subtilisin Crystals

We prepared subtilisin crystals (30-40 μm average, 27 mg/ml in Na_2SO_4) as previously described for subtilisin crystallization.

We then crosslinked the crystals using one of the following crosslinkers:

1) Dimethyl 3, 3'-dithiobispropionimidate•HCl - (DTBP) (Pierce)
2) Dithiobis(succinimidylpropionate) - (DSP)(Pierce)
3) 3, 3'- Dithiobis (sulfosuccinimidylpropionate) - (DTSSP) (Pierce).

Crosslinking was carried out in 15 ml neoprene screw cap tubes by placing 740 μl of subtilisin crystal slurry (20 mg) in 9.26 ml of buffer (25 mM $NaCO_3$/50 mM $NaHCO_3$, pH 8.0). One crosslinker was

1. (Twice amended) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that:

a) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

b) said protein crystal [being] is capable of controlled dissolution from insoluble and stable form to soluble and active form and releasing between about 0.1% and about 100% of crystalline protein as soluble protein per day upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.

2. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said change from concentrate to dilute form comprises dilution of said protein crystal in a solution [a change in solute concentration].

3. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution [change in solute concentration] comprises an increase or decrease in salt concentration.

4. (Amended) The crosslinked protein crystal according to claim 3, wherein said dilution of said protein crystal in a solution [change in solute concentration] comprises a decrease in salt concentration.

5. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution [change in solute concentration] comprises an increase or decrease in water concentration.

6. (Amended) The crosslinked protein crystal according to claim 5, wherein said dilution of said protein crystal in a solution [change in solute concentration] comprises an increase in water concentration.

7. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution [change in solute concentration] comprises an increase or decrease in organic solvent concentration.

8. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution [change in solute concentration] comprises a decrease in detergent concentration.

9. (Amended) The crosslinked protein crystal according to claim 2, wherein said dilution of said protein crystal in a solution [change in solute concentration] comprises a decrease in protein concentration.

10. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said change from concentrate to dilute form comprises a change in concentration of all solutes from about 2-fold to about 10,000-fold.

12. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said change in pH comprises a change from acidic pH to basic pH.

14. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said change in temperature comprises an increase or decrease in temperature.

16. (Amended) The crosslinked protein crystal according to any one of claims 1, or 86-88, wherein said active form of said protein is a form which is active against macromolecular substrates.

17. (Twice amended) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that:

a) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

b) said protein crystal [having] has a half-life of activity under storage conditions which is greater than at least 2 times that of the soluble form of the protein that is crystallized to form said crystal that is crosslinked and activity similar to that of the soluble form of the protein under conditions of use and which releases between about 0.1% and about 100% of crystalline protein as soluble protein per day.

18. (Twice amended) A crosslinked protein crystal, said protein crystal being crosslinked by a multifunctional crosslinking agent such that:

a) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart protein crystal when stored in phosphate buffered saline solution at 30°C for between about 100 and about 350 hours; and

b) said protein crystal [being] is capable of releasing its protein activity at a controlled rate of between about 0.1% and about 100% of crystalline protein as soluble protein per day upon exposure to a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in pH, change in solute concentration, change in temperature, change in chemical composition, change in shear force acting upon the crystal[s] and combinations thereof.

19. (Amended) The crosslinked protein crystal according to claim 18, wherein said controlled rate of releasing protein activity is determined by a factor selected from the group consisting of: the degree of crosslinking of said crosslinked protein crystal, the length of time of exposure of protein crystal to the crosslinker, the amino acid residues involved in the crosslinks, whether the crosslinker is homobifunctional or heterobifunctional, the rate of addition of the crosslinking agent to said protein crystal, the nature of the crosslinker, the chain length of the crosslinker, the surface area of said crosslinked protein crystal, the size of said crosslinked protein crystal, the shape of said crosslinked protein crystal and combinations thereof.

23. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, said protein crystal being substantially insoluble and stable in a composition under storage conditions and

substantially soluble and active under conditions of use of said composition.

27. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, wherein said protein is an enzyme.

30. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, wherein said protein is selected from the group consisting of therapeutic proteins, cleaning agent proteins, personal care proteins, veterinary proteins, food proteins, feed proteins, diagnostic proteins and decontamination proteins.

31. (Twice amended) The crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, wherein said protein is selected from the group consisting of hormones, antibodies, inhibitors, growth factors, trophic factors, cytokines, lymphokines, [toxoids,] growth hormones, nerve growth hormones, bone morphogenic proteins, toxoids and nutrients.

32. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, wherein said protein is selected from the group consisting of insulin, amylin, erythropoietin, Factor VIII, TPA, dornase-α, α-1-antitripsin, urease, fertility hormones, FSH, LSH, postridical hormones, tetanus toxoid and diptheria toxoid.

33. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, said crystal having a longest dimension of between about 0.01 μm and about 500 μm.

34. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, 18 or 86-88 [20 or 21], said crystal having a longest dimension of between about 0.1 μm and about 50 μm.

35. (Amended) The crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, said crystal having a shape selected from the group consisting of: spheres, needles, rods, plates, rhomboids, cubes, bipryamids and prisms.

36. (Amended) A composition comprising a crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, said composition being selected from the group consisting of cleaning agents, detergents, personal care compositions, cosmetics, pharmaceuticals, veterinary compounds, vaccines, foods, feeds, diagnostics and formulations for decontamination.

39. (Twice amended) A protein delivery system, said system comprising crosslinked protein crystals according to any one of claims 1, 17, [or] 18, or 86-88, and a delivery device.

45. (Amended) A detergent formulation comprising a crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88.

46. (Amended) A controlled release formulation comprising a crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88.

47. (Amended) A pharmaceutical controlled release formulation comprising a crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88.

52. (Amended) A vaccine comprising a crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88.

53. (Amended) A formulation comprising a crosslinked protein crystal according to any one of claims 1, 17, [or] 18, or 86-88, said formulation being selected from the group consisting of tablets, liposomes, granules, spheres, microspheres, microparticles and capsules.

54. (Twice amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals:

a) are between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

b) are characterized by the ability to change from insoluble and stable form to soluble and active form upon a change in their environment, and to release between about 0.1% and about 100% of crystalline protein as soluble protein per day, wherein said change [being] is selected from the group consisting of change in temperature, change in pH, change in chemical composition, change from

concentrate to dilute form, change in shear force acting upon the crystals and combinations thereof.

55. (Twice amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals are:

a) characterized by a half-life of activity under storage conditions which is greater than at least 2 times that of the soluble form of the protein that is crystallized to form said crystals that are crosslinked; and

b) between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours; and

c) characterized by an activity similar to that of the soluble form of the protein and which releases between about 0.1% and about 100% of crystalline protein as soluble protein per day under conditions of use.

56. (Twice amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals are:

a) characterized by being capable of releasing their protein activity at a controlled rate and which release between

about 0.1% and about 100% of crystalline protein as soluble protein per day upon exposure to a change in their environment, said change being selected from the group consisting of: change in pH, change in soluble concentration, change in temperature, change in chemical composition, change in shear force acting upon the crystals and combinations thereof; and

b) said protein crystal is between about 30% and about 80% as soluble as an uncrosslinked counterpart of said protein crystal when stored in phosphate buffered saline solution at about 30°C for between about 100 and about 350 hours.

84. (Amended) The method for producing crosslinked protein crystals according to claim 83, wherein said protein is selected from the group consisting of hormones, antibodies, inhibitors, growth factors, trophic factors, cytokines, lymphokines, [toxoids,] growth hormones, nerve growth hormones, bone morphogenic proteins, toxoids, vitamins and nutrients.

Please add claims 86-88.

86. (New) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form and releasing about 100% of crystalline protein as soluble protein per day upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.

87. (New) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional

crosslinking agent such that said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form and releasing about 100% of crystalline protein as soluble protein per hour upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.

88. (New) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent such that said protein crystal is capable of controlled dissolution from insoluble and stable form to soluble and active form and releasing between about 1% and about 50% of crystalline protein as soluble protein per minute upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.